IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/986,234 Confirmation No.: 4995

Applicant : Lazar, M.

Filed : October 22, 2001

TC/A.U. : 1644

Examiner : Ewoldt, G.

Customer No. : 00270

Title : COMPOSITIONS, METHODS, AND KITS RELATING TO RESISTIN

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR §1.132

Sir:

I, Mitchell A. Lazar, M.D., Ph.D., residing at 1008 Stony Lane, Gladwyne, PA, 19035, a citizen of United States, do declare and state:

1. I am the named inventor of the claims in the above-identified patent application, and of the claims now pending.

2. I submit this Declaration in the above-identified application to provide comments in connection with the response and/or amendment filed herewith pursuant to the Office Action dated January 18, 2006 for the above-identified patent application, and particularly to comment on the scientific merits of the documents cited by the Examiner in the rejection for lack of enablement of the claimed invention.

3. On information and belief, the Examiner's contention is that the above-referenced information in the specification does not enable use of antibodies to resistin in the treatment of type II diabetes in ***humans***. However, as described further herein, the Examiner's reliance on Heilbronn *et al*, J. Clin. Endocrin. Met., **2004**, 89(4):1844-1848; Volarova de Courten *et al*, Diabetes, **2004**, 53:1279-1284; Iqbal *et al*, Eur. Rev. Med. Pharmacol. Sci., **2005**, 9:161-165 and Lee *et al*, J. Clin. Endocrin. Met., **2003**, 88(10):4848-4856 in support of this contention is misplaced. I declare to the best of my knowledge and scientific expertise that these documents are inadequate to support this contention. Moreover, the conclusions reached by these documents are contrary to the conclusions reached by numerous other documents which support the role of resistin levels in human type II diabetes. On information and belief, some combination of sample size, population being studied, assay employed and actual performance of the assays may be related to these conclusions. The references cited by the Examiner are discussed below individually.

4. Heilbronn *et al*'s 2004 publication asserts that their study is the first assessment in human subjects of serum resistin and insulin sensitivity by the insulin clamp technique. Heilbronn *et al* studied the relationship between serum resistin and insulin sensitivity, fat distribution, fat cell size and ectopic fat deposition in a wide variety of subjects, including subjects with type II diabetes (page. 1844, col. 2). Heilbronn *et al.* disclose results showing that there is a statistically significant ($p \leq 0.05$) relationship between serum resistin level and glucose disposal rate (p. 1846: Fig. 1). Accordingly, contrary to the Examiner's contention, these results support the role of resistin in glucose homeostasis in humans. This reference does not duplicate those findings when evaluated in diabetic individuals. The authors state: *"However, serum resistin was not different in . . . diabetic groups despite wide variations in insulin sensitivity"*, citing pp. 1846-1874. This study also used the Biovendor assay that was questioned in the later Iqbal *et al* study, as further discussed below. Heilbronn *et al* also cautioned that its results:

> *"**imply** that resistin does not cause peripheral insulin resistance in humans, **at least not through** impaired adipocyte proliferation/differentiation or increasing ectopic fat deposition."* Page 1847, col. 1, emphasis added.

Heilbronn *et al* also acknowledged possible reasons for result variation in the composition of its studied populations and acknowledged that other publications found different results. See page 1847, col. 2. Thus, contrary to the Examiner's contention, Heilbronn *et al* actually supports the contention that serum resistin plays a role in glucose homeostasis, and does not clearly address or contradict our teachings regarding the use of anti-resistin antibodies in the treatment of Type 2 diabetes.

5. Volarova de Courten *et al*'s 2004 study subjected a population of Pima Indians to a protocol including a weight-maintaining diet, followed by a 12 hour fast, followed by a 2 hour glucose tolerance test. Plasma insulin levels were measured by an automated immunoassay (BioVender Laboratory). The study results purportedly showed that there *is* a significant relationship between serum resistin levels and serum glucose levels two hours after a glucose load (p. 1281: Fig. 1C). After adjusting for percent body fat, these authors state that there was no association (see, e.g., p. 1282: col. 1, ¶ 2). However, the authors also state that "higher serum resistin levels were associated with adiposity" (p. 1273, col. 1, ¶ 2). This study also cautions that,

> "the true nature of our observation remains largely unexplained and that caution should be exercised in drawing definitive conclusions" (see p. 1283).

Thus, contrary to the Examiner's contention, Volarova de Courten *et al* does not clearly demonstrate or support the contention that serum resistin levels have no effect on serum glucose levels, and further does not clearly address or contradict our teachings regarding the use of anti-resistin antibodies in the treatment of Type 2 diabetes.

6. Iqbal *et al*'s 2005 publication report findings related to weight loss and serum resistin concentrations, which findings were obtained from an *ad hoc* analysis of a patient population being studied for other reasons. Thus, Iqbal *et al*'s conclusion that no correlation between resistin and type II diabetes was demonstrated (see pp. 161 and 164) is questionable. Iqbal *et al* clearly concedes that

> "Given the newness of the ELISA assay, the restricted population, and the unknown kinetics of resistin, more studies are needed before the role of resistin in insulin sensitivity and obesity can fully be defined." (See p. 164).

In addition, the study was underpowered. To illustrate this, the standard error in one of the resistin measurements was so great, more than 27 times the mean value, that significant changes could not possibly be detected with 32 or 39 subjects in each group (p. 163: Fig. 2). This may be an indicator of issues with the assay, which was acknowledged by the authors (p. 164: column 2, ¶ 2). It is further noted that the study was extremely restrictive in scope and patient population, which may be yet another reason for any results that conflict with our findings, *e.g.* the patient population was composed of only morbidly obese individuals, *i.e.* a BMI $\geq$ 35 kg/m^2, (p. 162: column 1, 1). Further, without any control, e.g., normal individuals, or mildly obese, the questions of whether glucose homeostasis in morbidly obese individuals is already abnormal is not addressed here.

7. Lee *et al*'s 2003 study involved anthropomorphic, metabolic and hormonal predictors of serum resistin levels in women and healthy young subjects, and then compared serum resistin levels in a separate study of 19 normal-weight healthy adolescents and 19 adolescents with insulin resistance to determine whether resistin levels are higher in insulin-resistant states. Lee *et al* refers to their study as one of the first human studies on circulating resistin levels (page. 4853, col. 1) and used what appears to be the same Biovendor Laboratory ELISA bioassay that was subject to question in the Iqbal *et al* study, discussed below. See page 4848, col. 1. Lee *et al.* states that there is no statistically *significant* difference between obese nondiabetic subjects (5.8 +/- 1.6 ng/mL), obese diabetic subjects (7.8 +/- 4.6 ng/mL), and normal-weight subjects (5.6 +/- 1.9 ng/mL). However it is noted that the standard error of the obese diabetic subjects is more than half of the mean value, which, as discussed in Iqbal *et al.*, may indicate that the study was underpowered.

8. In my opinion and on information and belief, the references that the Examiner has relied on are inadequate to support any contention that human resistin does not operate in type II diabetes in the same manner as mouse resistin does in mice. As discussed above, these documents relied on by the Examiner, are themselves inconclusive as to the effects of resistin on glucose homeostasis. Further, to the extent that the documents relied upon

by the Examiner as contradictory to this invention, it is my opinion and belief that these documents represent the minority of documents in the field of resistin and diabetes.

9. On information and belief, a majority of documents in the art do support the role of resistin in type 2 diabetes in humans. See, for example, McTernan *et al*, **2003** *J. Clin. Endocrinol. Metab.* 88:6098-6106 and Youn *et al*, **2004** J. Clin. Endocrinol. Metab., 89: 150-156 report that serum resistin is elevated in human type II diabetes, thus contradicting the conclusions of Lee *et al*, cited above. Similarly, Fujinami *et al* **2004** *Clin. Chim. Acta*, 339:57-63 states that "[t]he mean resistin concentration in diabetic patients . . . was significantly higher than that in age-, sex- and BMI- matched normal subjects." (See pp. 60 and 61). Azuma *et al*, **2003** *Obes. Res.*, 11:997-1001 reported that serum resistin levels are increased in obesity.

10. In additional examples, Osawa H. *et al*, **2004** "The G/G Genotype of a Resistin Single-Nucleotide Polymorphism at -420 Increases Type 2 Diabetes Mellitus Susceptibility by Inducing Promoter Activity through Specific Binding of Sp1/3" *Am J Hum Genet* 75:678-686; Osawa, H *et al*, **2005** "Resistin SNP-420 determines its monocyte mRNA and serum levels inducing type 2 diabetes." *Biochem Biophys Res Commun* 335:596-602 relate to studies that disclose that a polymorphism in the resistin promoter (-420 C=>G) is associated with type II diabetes susceptibility and is associated with obesity or insulin resistance in humans. Results demonstrate this polymorphism in the resistin promoter leads to over-expression of the resistin gene and consequently increased resistin levels. This increase is "gene-dosage" dependent, in that heterozygotes had a 50% increase in level and homozygotes for the polymorphism had a 100% increase (double). And, the patients with increased resistin levels had very significantly increased risk of type 2 diabetes. Importantly, this was by far the biggest study to date (397 cases and 406 controls; $p = 0.008$; adjusted odds ratio = 1.97). Furthermore, the conclusions held up in a meta-analysis of 1,888 cases and 1,648 controls. These authors also cite Smith *et al*, **2003** *Diabetes*, 52:1611-1618 as reporting that resistin mRNA levels are higher in adipose tissues of obese human subjects with the SNP-420 G/G genotype. Zhang et al, **2003**, *Diabet. Med.*, 20(10):828-31 investigated the relationship between

serum resistin levels and blood glucose. The authors found that fasting serum resistin concentrations were higher in the type II diabetes subjects than normal glucose tolerance subjects and concluded that resistin may be involved in diabetes in humans.

11. In a review paper authored by C. M. Steppan and me, i.e., Steppan and Lazar, **2004** *J. Internal Medicine*, 244:439-447, we acknowledged that there was no clear consensus about the biological role of resistin physiology, but observed that, at least in some human populations, polymorphisms at the resistin locus are associated with obesity and/or insulin sensitivity.

12. In still another more recent publication, i.e., Al-Harithy and Al-Ghamdi, **2005** Ann. Saudi Med., 24(4):283-7 studied the relationship between serum resistin concentrations and insulin resistance in lean, overweight and obese non-diabetic and diabetic Saudi women. The authors concluded that "Resistin concentrations are elevated in patients with type 2 diabetes and are associated with obesity and insulin resistance." They concluded that significantly higher levels of resistin were observed in diabetic compared to the lean and non-diabetic subjects.

13. Further, in yet another example, a very recently issue electronic publication, i.e., Menzahi *et al*, **2006** *J Clin Endocrinol Metab.* [Epub ahead of print] entitled "Heritability of serum resistin and its genetic correlation with insulin resistance-related features in non-diabetic Caucasians", the authors studied 264 non diabetic probands, Caucasian from Italy, and their 473 adult family members. The data indicated that serum resistin is highly heritable and has some common genetic background with traits related to insulin resistance, reinforcing the hypothesis that this adipokine may play a pathogenic role in insulin resistance-related abnormalities, including type 2 diabetes and cardiovascular disease.

14. Three additional publications show that antidiabetic thiazolidinedione drugs reduce resistin levels in humans, which is the same relationship as stated in the above-identified patent application between murine resistin in mice treated with these drugs, i.e.,

that these drugs reduced murine resistin levels in mice. For example, Bajaj M, *et al.*, Int J Obes Relat Metab Disord., **2004** Jun 28(6):783-9 concludes that the antidiabetic thiazolidinedione (TZD) pioglitazone significantly reduces serum resistin levels in humans with type 2 diabetes. Szapary PO *et al*, Arterioscler Thromb Vasc Biol. **2006** Jan;26(1):182-8 asserts that the antidiabetic thiazolidinedione pioglitazone significantly reduces resistin levels in humans with obesity and metabolic syndrome. Finally, Samaha FF *et al*, Arterioscler Thromb Vasc Biol. **2006** Mar;26(3):624-30 indicates that the antidiabetic TZD rosiglitazone significantly lowers resistin levels in humans with metabolic syndrome.

15. On information and belief, as demonstrated above, the weight of the scientific evidence to date supports the conclusions drawn in the original specification that human resistin, like mouse resistin, has a diabetogenic effect, at least in some populations of humans, and that altering resistin concentrations *in vivo* represents a viable therapeutic option for treating or alleviating type II diabetes in those populations demonstrating this effect.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: May 17, 2006 By: Mitchell A. Lazar, M.D., Ph.D.